



Patent
Attorney's Docket No. 14149-4US FC/VC/

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Louis-Philippe Vézina et al. EXAMINER:

SERIAL NO.: 09/678,303 ART UNIT:

FILED: October 3, 2000

FOR: PROMOTER FOR REGULATING EXPRESSION OF FOREIGN GENES

PRELIMINARY SUBMISSION

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

In order to expedite the prosecution of the present application, please consider the following submission.

REMARKS

Claims 1-8 are still in the application.

Affidavit Declaration

Submitted herewith is a Declaration of Dr. Marc-André Daoust to provide additional results of the method of the present application for Alfalfa and tobacco leaves transformation using particle bombardment.

The present invention presents unexpected results in view of the prior art, in that no one skilled in the art could have imagine a method to generate transgenic alfalfa lines that can be regulated in their expression of a reporter gene.

Substantive examination of the application and of the claims is earnestly solicited.

No fees are believed to be necessitated by this preliminary submission. However, should this be an error, authorization is hereby given to charge Deposit Account No. 19-5113 for any underpayment or to credit any overpayment.

In the event that there are any questions concerning this submission, or the application in general, the Examiner is respectfully urged to telephone the undersigned so that prosecution of this application may be expedited.

Respectfully submitted,



France Côté
Registration No. 37,037

Date: February 14, 2001

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Patent
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Medicago inc.

EXAMINER:

SERIAL NO.: 09/878,303

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FOR: PROMOTER FOR REGULATING EXPRESSION OF FOREIGN
GENES

DECLARATION OF Dr. Marc-André DAOUST

I, Marc-André DAOUST hereby declare and say:

1. I am a citizen of Canada, presently residing at 939, Avenue de
Manrière App. 2, Québec, Québec, Canada.

2. I am a team leader at Medicago inc., the owner of the above-
identified patent.

3. That my academic background and experiences in the field of
the present invention are listed on the enclosed *curriculum vitae*.

4. I am one of the inventors in the present application and have
read and understand the specification.

5. That we have conducted an experiment that supports the subject matter of the present application. Methods and results of this experiment are reproduced thereto:

Alfalfa and tobacco leaves transformation using particle bombardment

Materials and Methods

The leaves used for transformation were collected from greenhouse-grown plants (tobacco) or from *in vitro* grown plants (alfalfa). For tobacco, one leaf disk, cut from a fully expanded young leaf, was used as explant whereas for alfalfa, three leaves were placed side by side in order to cover a maximum area. Twenty four hours prior to bombardment, the leaves were sterilized, placed on solid MS medium with the abaxial face up and kept at 25°C. The bombardment was performed using a Biolistic PDS-1000/He particle delivery system (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer specifications. Following bombardment, the leaves were left on solid MS culture medium for 24 h before being transferred into a GUS staining solution, and placed at 37°C overnight. The number of blue cells was counted after washing the chlorophyll with 70% ethanol.

Results

Transient expression of the GUS gene was used to demonstrate the activity of the promoter in tobacco and alfalfa leaves. Data presented in table 1 show that for both types of leaves, the promoter efficiently drove GUS expression. Furthermore, using the P960 deletion, the number of blue cells per bombardment was 3-fold (alfalfa) and 9-fold (tobacco) higher than when using the CaMV 35S

promoter. These results demonstrate the capacity of the regulatory sequences found in pGPlas3-2 to act as an efficient promoter when placed operationally upstream of a coding sequence.

Table 1
Comparison of promoter efficiency in leaves

Plant	Promoter activity (number of blue cells per bombardment)*		
	P729	P960	368
Tobacco	2	28	3
Alfalfa A1.93	17	115	34
Alfalfa 11.9	21	90	31

* Each value represents the mean number of blue cells from 3 bombardments.

6. I declare further that all statements made on information and belief are believed to be true, and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and such willful false statements may jeopardize the validity of the instant patent specification or any patent issuing thereon.

Date: 29 January 2001

By: John Doe



Marc-André D'Aoust

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EDUCATION

1990-1993 Bachelor in Biology (B.Sc)
Specialization : Plant Biology
Université Laval, Québec, Canada

1993-2000 Master and Ph.D. in Plant Molecular Biology / Plant Physiology
Université Laval, Québec, Canada
Thesis title: Study of the tissue-specific expression of sucrose synthase in tomato and the physiological role of the pericarp specific isoform using antisense inhibition

EXPERIENCES

2000- Medicago, Sainte-Foy, Canada
Team Leader
Development of expression systems and supervision of the expression cassette construction team under the supervision of Dr. Louis Vézina (Scientific Director).

1999- Medicago, Sainte-Foy, Canada
2000 **Project Manager**
Coordination of the research activities for the plant expression vectors team under the supervision of Dr. Louis Vézina (Scientific Director).

1993- Université Laval, Canada
2000 **Master and Ph.D. student**
Construction of plant synthetic genes, transformation, and analysis of transgenic tomato plants.
Direction of Dr. Serge Yelle and co-direction of Dr. Binh Nguyen-Quoc.

1994- Université Laval, Canada
1995 **Teacher of plant physiology laboratory**
Direction of Dr. Serge Yelle and supervision of Monique Dufour.

1993 Université Laval, Canada
Research assistant
Direction of Dr. Serge Yelle and supervision of Dr. Binh Nguyen-Quoc.

HONORS

1998 **Chairman and invited speaker** at the carbon partitioning minisymposium of the annual meeting of the American Society of Plant Physiology (ASPP). Madison, WI, USA.

1996-1998 **Research scholarship** from the "Fondation de l'Université Laval", Université Laval, Québec, Canada.

ACTIVITIES

1993-1994 President of the association of graduate students in Plant Science (Department). Université Laval, Québec, Canada.

1993-1994 Vice-president of the association of graduate students in Agriculture and Food Science (Faculty). Université Laval, Québec, Canada.

PUBLICATIONS

M. Dorais, B. Nguyen-Quoc, H. N'tchobo, M.-A. D'Aoust, C. Foyer, A. Gosselin and S. Yelle (1998). *What controls sucrose unloading in tomato fruits?* Acta Horticulturae 487: 107-113.

M.-A. D'Aoust, B. Nguyen-Quoc, V.-Q. Le and S. Yelle (1999). *Upstream regulatory regions from the Sh1 promoter confer tissue-specific expression of the GUS gene in tomato.* Plant Cell Report 18: 803-808.

M.-A. D'Aoust, B. Nguyen-Quoc and S. Yelle (1999). *Antisense inhibition of sucrose synthase reveals its function in the control of fruit setting and development in tomato.* Plant Cell 11: 2407-2418.

MEETING COMMUNICATIONS : ORAL PRESENTATIONS

M.-A. D'Aoust, B. Nguyen-Quoc and S. Yelle (1998). *Starch metabolism in tomato fruit under sucrose synthase antisense inhibition.* Annual meeting of the Canadian Society of Plant Physiologists (CSPP). July 11-14 in Montréal, Québec, Canada.

M.-A. D'Aoust, B. Nguyen-Quoc and S. Yelle (1998). *Sucrose import and metabolism in fruits of transgenic tomato with reduced sucrose synthase activity.* Carbon partitioning minisymposium. Annual meeting of the American Society of Plant Physiologists (ASPP). June 27- July 1, Madison, Wisconsin, USA.

M.-A. D'Aoust, B. Nguyen-Quoc, V.-Q. Le and S. Yelle (1998). *Mise en évidence du rôle exclusif de la saccharose synthase du fruit de tomate par des mutants antisens TOMSSF.* Symposium on the control and physiology of photosynthesis. Annual meeting of the "Association Canadienne Française pour l'Avancement des Sciences" (ACFAS). May 11-15, Québec, Québec, Canada.

M.-A. D'Aoust, B. Nguyen-Quoc, V.-Q. Le, X.-F. Huang and S. Yelle (1996). *Interactions between regulatory regions of the maize Sh1 gene and the truncated 35S promoter results in a strong endosperm-specific promoter in tomato.* Annual meeting of the American Society of Plant Physiologists (ASPP). July 27-31, San Antonio, Texas, USA.

M.-A. D'Aoust, B. Nguyen-Quoc, X.-F. Huang and S. Yelle (1994). *Construction d'un promoteur synthétique à fort niveau d'expression et possédant la spécificité tissulaire du promoteur de la saccharose synthase (Sh1).* Annual meeting of the "Association Canadienne Française pour l'Avancement des Sciences" (ACFAS). May 16-20, Montréal, Québec, Canada.

POSTER PRESENTATIONS

B. Nguyen-Quoc, M.-A. D'Aoust, H. N'tchobo and C. Foyer (1999) *The roles of sucrose synthase, sucrose phosphate synthase and invertase in the regulation of sucrose import into developing tomato fruit.* International Conference on Assimilate Transport and Partitioning (ICATP). August 15-20, Newcastle, Australia.

M.-A. D'Aoust, S. Yelle and B. Nguyen-Quoc (1999). *Sucrose synthase regulates fruit setting and development in tomato.* Annual meeting of the Canadian Society of Plant Physiologists (CSPP). June 19-23, Saskatoon, Canada.

M.-A. D'Aoust, B. Nguyen-Quoc and S. Yelle (1998). *Biochemical analysis of transgenic tomato plants expressing sucrose synthase antisense RNA.* Annual meeting of the American Society of Plant Physiologists (ASPP). June 27-July 1, Madison, Wisconsin, USA.

M.-A. D'Aoust, B. Nguyen-Quoc, X.-F. Huang, R. Anguenot, N. Daly, V.-Q. Le and S. Yelle (1995). *Regulation of sucrose synthase genes: Comparison of their expression in maize and tomato plants.* First International Symposium on Sucrose Metabolism (FISSM). May 7-13, Mar del Plata, Argentina.